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14. ABSTRACT Accumulating evidence points to a role of chronic inflammation in the pathogenesis and progression of cancers, including prostate. Infections are important agents in the genesis of inflammation. For prostate cancer, several lines of evidence point to a role of infections as important agents, although no specific infection has consistently been identified. In this project, we are examining two specific infectious agents with respect to prostate cancer: <i>T. vaginalis</i> , the most common non-viral sexually transmitted infection, and the recently identified retrovirus XMRV. The aims of this study are 1-) To assess the role of the newly identified XMRV virus in prostate carcinogenesis and progression; 2-) To characterize the role of the infectious protozoa <i>T. vaginalis</i> in prostate carcinogenesis and progression. The current study is nested within the Swedish Watchful Waiting Cohort, a population-based cohort of 1,256 Swedish men diagnosed with localized prostate cancer. During 28 years of follow-up, 320 men have died of cancer, and thus this is a powerful population in which to examine determinants of prostate cancer progression. A tumor repository from archival tissue specimens have been collected from all men in the cohort and will be used to assay for presence of the infections.					
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Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	5
Key Research Accomplishments.....	6
Reportable Outcomes.....	7
Conclusion.....	8
References.....	9
Appendices.....	10

Introduction

Prostate cancer has considerable biologic heterogeneity, such that some men experience an aggressive course while many have a slow growing or indolent disease (1-3). Thus, central issues in prostate cancer research are to identify mechanisms which are amenable to prevention and treatment, and to understand pathways that lead to aggressive cancer.

A growing body of epidemiologic, genetic and molecular pathological data points to the role of chronic inflammation in the pathogenesis and progression of prostate cancer (4). The pathways involved in chronic inflammation induce cellular damage and compensatory cellular proliferation (5). Clinical prostatitis, which occurs in approximately 9% of men between the ages of 40 and 79, has been linked to prostate cancer in several epidemiologic studies (6). Moreover, surgical prostate tumor specimens often exhibit histological evidence of prostatitis, although the determinants of this prostatic inflammation are unclear.

Perhaps of greater importance, data also suggests that the degree of inflammation may be a predictor of more aggressive disease. In a study of 161 men undergoing radical prostatectomy (7), 5-year recurrence-free survival was significantly lower among patients with high-grade inflammation in malignant tissue (27%) than in patients with low-grade or no evidence of inflammation (65%), independent of Gleason grade, preoperative PSA level, and pathologic stage.

Infectious agents are likely targets involved in the initiation and exacerbation of chronic inflammation, and infections can lead to increased risk of several cancers (8). Indeed, an estimated 15% of malignancies globally are thought to have an infectious etiology (9, 10). Infectious agents may also have direct effects on carcinogenesis through the transformation of cells via incorporation of active oncogenes into the host genome, inhibition of tumor suppressors, stimulation of proliferation signals, or through immune suppression. Known oncogenic infections are typically highly prevalent within the host population, persistent within the host, and require a variety of co-factors for malignant transformation.

Two recent papers (11, 12) provide emerging evidence suggesting involvement of the newly identified murine-like retrovirus XMRV and the protozoan *T. vaginalis* in prostate cancer; these are the focus of the proposed study. The objective of the proposed study is to evaluate and extend the initial findings on *T. vaginalis* and XMRV, and to more fully characterize the potential role of these infections in the pathogenesis and progression of prostate cancer in a large population-based cohort of Swedish men with prostate cancer who have been followed prospectively for 28 years.

Body

Aim I. To assess the role of the newly identified XMRV virus in prostate carcinogenesis and progression.

We have completed abstraction of clinical data from medical records and pathology reports for the 678 prostate cancer cases in the Swedish Watchful Waiting Cohort. We have retrieved information on tumor grade, stage, extent, PSA levels at diagnosis, clinical presentation and development of metastases. The abstraction of clinical parameters is complete. A clinical database in SAS has been constructed including the cases for which abstraction of clinical parameters is complete. Quality control assessment of this database is currently underway, and we have shown high quality of the data.

We have now retrieved tumor tissue specimens for all 678 of the men. The blocks have been reviewed histologically by our pathologist for TMA construction. We have completed construction of three tissue microarrays (TMAs), as well as a test array to be used for pilot studies of the biomarkers, and were plan another 3 arrays for the study.

The pathologist has also completed 50% review of cases for circling areas of benign tissue on the tumor blocks for extraction of DNA for the characterization of RNASEL genotype. In preliminary work, we have shown excellent yields of DNA (100-200 ng) from 3 cores of benign tissue, which will be more than sufficient for our genotyping assays.

Our pathologist has also completed review of the cases for evidence of the preneoplastic proliferative inflammatory atrophy lesions. These data have been entered into an ACCESS database, indicated by a Research ID, and the data are in the process of being cleaned now.

We have had two working meetings with our colleagues at the Cleveland Clinic regarding the tumor tissue assays for XMRV. We are going to be initiating pilot studies on the test array during the next couple of months.

Aim II. To characterize the role of the infectious protozoa *T. vaginalis* in prostate carcinogenesis and progression.

Much of the preliminary work summarized in Aim I is directly relevant to Aim II of the project, including the clinical data review, tissue retrieval, histologic evaluation, TMA construction, and PIA assessment.

Relevant for this aim, we have also undertaken pilot work on our test array to work up antibody concentrations for the T Vaginalis antibody, that was retrieved from the lab of Dr. Alderete. The pathologist is reviewing those slides now. In addition, we are also in the process of working with clinical colleagues to identify positive controls to be used for this project, namely known T vaginalis positive samples.

Key Research Accomplishments

- Developed Clinical database for 648 prostate cancer cases in the Swedish Watchful Waiting Cohort
- Assembled tumor tissue repository of TURP specimens for 678 cases, including tracking system
- Finished standardized histopathologic review of tumor tissue cohort and constructed 3 tumor tissue microarrays and 1 test array
- Evaluated tissue specimens for presence of PIA lesions
- Undertook pilot studies of T vaginalis
- Extracted DNA from benign tissue in pilot study of 92 cases and found excellent DNA yields
- Organized two planning meetings for analyses of XMRV

Reportable Outcomes

- Recruited post-doctoral fellow to start working on the project
- Recruited collaborator Dr. Katja Fall from Karolinska Institutet as Visiting Scientist at Harvard School of Public Health for next two years
- Invited as faculty speaker at the Prouts Neck Prostate Cancer Meeting to be held November 2008
- Development of prostate tumor tissue repository of TURP specimens
- Received Milton Fund Award from Harvard University related to this work
- Data generated from this project will provide thesis data for one doctoral student at Harvard School of Public Health
- PI was promoted to Assistant Professor of Medicine at Harvard Medical School and Assistant Professor of Epidemiology at Harvard School of Public Health based on experience supported by this award

Conclusion

We are in the preliminary stages of the project. However, we have demonstrated our ability to undertake this large cohort and collect archival tumor specimens from 678 men. We have demonstrated a proven working relation with the pathology team, as shown by progress on construction of the tissue microarrays and evaluation of PIA lesions. During the next year, we will complete the biomarker analyses and undertake statistical analyses to successfully complete the study aims. Ultimately, this project has the potential to provide strong evidence (for or against) in assessing the role of infectious agents and inflammation in prostate pathogenesis. Moreover, the tumor tissue repository we are establishing is a unique resource in which to test future hypothesis. Given the substantial biologic heterogeneity of prostate cancer, the proposed project would ultimately have exciting implications for prevention and potentially treatment of prostate cancer.

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Appendices

None